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Effects of natriuretic peptide receptor inhibition on remnant kidney function in rats

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Effects of natriuretic peptide receptor inhibition on remnant kidney function in rats. To identify the contribution of natriuretic peptide (NP) activity to the adaptative increases in glomerular filtration rate (GFR), effective renal plasma flow rate (ERPF) and fractional sodium excretion (FE_{Na}) observed in the remnant kidney, we investigated the acute effects of administering HS-142-1 (HS), a potent NP receptor antagonist, in 5/6th nephrectomized (NPX) rats. In addition to normal sodium intake, high or low sodium intakes were used to stimulate or suppress, respectively, endogenous NP activity in NPX rats. In rats three days after NPX on high sodium, HS (20 mg/kg bolus i.v.) reduced GFR from 0.55 ± 0.05 to 0.35 ± 0.04 ml/min; ERPF from 1.83 ± 0.19 to 1.53 ± 0.16 ml/min; and FE_{Na} from 7.1 ± 1.1 to 1.6 ± 0.4%, without affecting MAP. Similar changes of lesser magnitude were observed in NPX rats on normal sodium intake. By contrast, GFR, ERPF, FE_{Na} and MAP were unchanged following HS in NPX rats on low sodium intake, suggesting that the magnitude of responses to HS is dependent upon the expected levels of activity of NP. We conclude that in anesthetized rats, natriuretic peptides contribute to the compensatory increases in GFR, ERPF and FE_{Na} observed in the remnant kidney under normal and salt-replete conditions.

As a compensatory response to reduced renal excretory capacity, rats subjected to 5/6 nephrectomy (NPX) excrete greatly elevated amounts of sodium and water per remnant nephron. This is effected by both increased filtered load of sodium, via increased glomerular filtration rate (GFR) per nephron, and decreased tubular reabsorption, as reflected in elevated fractional excretion of sodium (FE_{Na}) [1]. Previous studies from this laboratory suggest that atrial natriuretic peptide (ANP), released from the heart in response to plasma volume expansion, may mediate these adaptive renal responses, at least in part [2, 3]. ANP levels in NPX rats are elevated in proportion to the chronic dietary sodium intake [2, 4] and ANP infused into normal rats may promote natriuresis by decreasing tubule sodium reabsorption and, at higher doses, by increasing GFR and hence filtered sodium load [5]. Dietary sodium restriction in NPX rats has been demonstrated to reduce plasma ANP levels and to reduce fractional sodium excretion concomitantly [2]. Furthermore, administration of specific anti-ANP antiserum decreased FE_{Na} in NPX rats on high sodium diet [3]. The availability of a novel specific pharmacologic antagonist of natriuretic peptide receptors, HS-142-1 (HS), provides a more precise means of defining the role of endogenous natriuretic peptides in the functional adaptive responses of remnant kidneys. The purpose of this study was to establish the role of natriuretic peptides in mediating the adaptive hemodynamic and excretory responses in the remnant kidney by evaluating the acute renal responses to HS at different levels of chronic salt intake. In protocol A we first sought to confirm the reported effects [6] of HS administration on the diuretic and natriuretic responses to acute volume expansion in euvolemic anesthetized Münich-Wistar rats. In protocol B, we then assessed the effects of HS administration on the hemodynamic and excretory function of the remnant kidney under conditions of high, normal or low salt diet, at early and at later stages after partial renal ablation.

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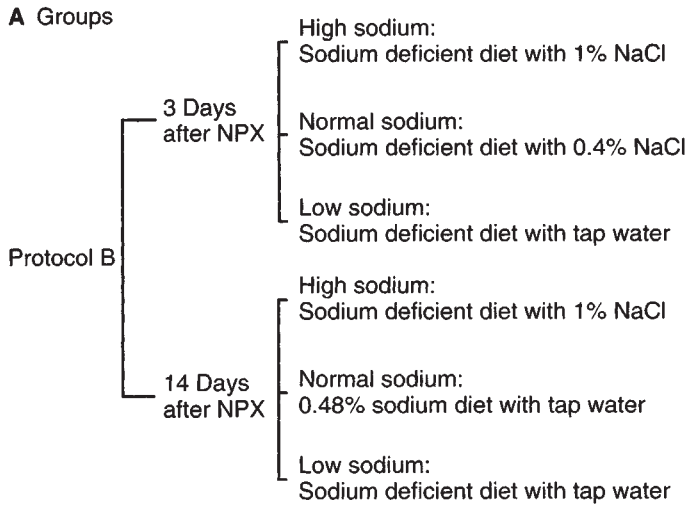
Methods

Adult male Münich-Wistar rats weighing from 220 to 270 g were used in the studies. Normal rats in protocol A received standard rat chow (Rodent Lab Chow 5001; Ralston Purina Co., St. Louis, Missouri, USA) and water *ad libitum*. In protocol B, 12 groups of rats underwent ablation of 5/6 of their total renal mass by surgical excision of the right kidney and infarction of approximately 2/3 of the left kidney by selective ligation of renal artery branches. Clearance experiments were carried out in rats either 3 or 14 days after partial nephrectomy (NPX) on high, normal or low sodium intakes. In the 3 day groups, NPX rats received high sodium (sodium deficient diet, ICN Biomedicals, Inc., Cleveland, Ohio, USA) with 1% NaCl water; normal (sodium deficient diet with 0.4% NaCl water) or low sodium (sodium deficient diet with tap water; Fig. 1A). In the 14 day groups, normal sodium intake was provided by standard rat chow with tap water whereas high and low sodium intakes were given as in the 3 day groups. Urine flow and sodium excretion rates were determined from 24-hour urine collections from individual rats in each group one to two days before the clearance experiments.

All rats were anesthetized with Inactin (0.1 g/kg, i.p.). Body temperature was maintained between 36 and 37°C by a thermostatically controlled heating table. After tracheostomy, the left femoral artery was cannulated for measurement of mean arterial blood pressure and intermittent blood sampling at the mid-point of each clearance period. The right femoral vein was cannulated for infusion of iso-oncotic rat plasma, volume equivalent to 0.8% of body weight, over 25 minutes to replace plasma loss due to anesthesia and surgery [7]. Subsequently, a sustained infusion of plasma at a rate of 0.6 ml/hr was given. In protocol A, a solution of



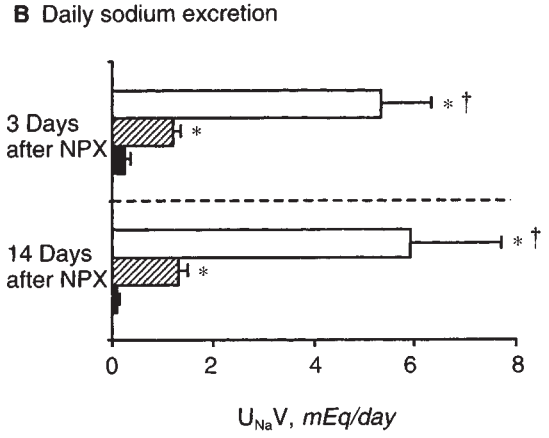


Fig. 1. A. *Assignment of groups of rats 3 or 14 days after 5/6 nephrectomy (NPX) on various levels of daily sodium intakes.* **B.** Daily sodium excretion from rats 3 or 14 days after 5/6 nephrectomy (NPX) at each level of sodium intake. Symbols are: (□) high sodium; (▨) normal sodium; (■) low sodium. *$P < 0.05$ vs. low sodium; †$P < 0.05$ vs. normal sodium.

8% inulin and 1% PAH in isotonic saline was infused continuously at a rate of 20 μl/min throughout each experiment. The left ureter was cannulated for collection of urine; the bladder (protocol A only) was cannulated to allow free drainage of urine from the right side. In protocol B, NPX rats on high and normal sodium intakes received continuous infusions of isotonic saline containing 4% inulin and 0.3% PAH at a rate of 20 μl/min (3.07 μEq Na^+ min), whereas NPX rats on low sodium intake were infused with the same solution at 14.2 μl (2.18 μEq Na^+ min) throughout the experiments. After a one hour stabilization period, two 15-minute baseline urine collections were made in all groups prior to the experimental interventions described below.

Protocol A

After two 15-minute baseline collections of urine, each rat received either an intravenous bolus dose of vehicle (0.15 ml isotonic saline) or HS (20 mg/kg) in 0.15 ml isotonic saline. Ten minutes later, acute volume expansion with isotonic saline (2% of body weight over 30 min) was commenced intravenously, during which two 15-minute urine samples were collected.

Protocol B

In all groups studied in protocol B, two baseline urine collections were followed by an intravenous bolus of vehicle or HS (20 mg/kg) in 0.15 ml isotonic saline. Ten minutes later, two 15 minute urine samples were collected.

Analysis

The concentrations of inulin and PAH were determined using the anthrone method [8] and a colorimetric technique [9], respectively. Urine volume was determined gravimetrically and GFR and effective renal plasma flow rate (ERPF) were assessed as clearances of inulin and PAH, respectively, calculated from standard formulae. The concentrations of sodium in both plasma and urine were measured by flame photometry.

Statistical analysis

All data are expressed as mean ± SEM. Differences between the baseline and experimental observations within groups were compared by Student's paired *t*-test. Comparisons between vehicle or HS treated groups were made using ANOVA followed by Sheffe's test where appropriate [10]. Significance was accepted at $P < 0.05$.

Results

Protocol A

Body weights in both groups were similar. Mean arterial blood pressure (MAP) was not significantly different between groups either before or after treatment (Fig. 2A). From similar baseline values for urine flow rate (V), acute volume expansion resulted in a threefold increase in V and a fourfold increase in sodium excretion rate ($U_{Na}V$) in vehicle-treated rats (Fig. 2 B, C). In rats receiving HS however, a less than 1.5-fold increase in V was observed. Remarkably, the increase in $U_{Na}V$ was almost completely abolished during acute saline loading in HS treated rats; similarly, whereas FE_{Na} in the vehicle group increased by more than threefold, FE_{Na} did not change significantly in the HS group (Table 1), indicating that the decrease in tubule sodium reabsorption induced by saline loading was abolished by the NP receptor antagonist. Likewise, the modest increase in GFR during volume expansion was suppressed in HS-treated rats (Table 1). The increase in ERPF during saline loading, however, was not completely suppressed in the HS group nor did HS prevent the modest reductions in hematocrit or filtration fraction (FF) seen during saline loading (Table 1).

Protocol B

Daily sodium excretion in NPX rats. Three days after NPX, daily $U_{Na}V$ (5.3 ± 1.0 mEq/day) in the high salt rats was significantly higher than in the normal and low sodium rats (1.2 ± 0.1 and 0.3 ± 0.1 mEq/day, respectively; Fig. 1B). Similarly, daily $U_{Na}V$ reflected the dietary intake of sodium in rats 14 days after NPX on high (5.9 ± 1.8 mEq/day), normal (1.3 ± 0.2) and low (0.1 ± 0.02) sodium diet.

Acute clearance studies. Studies were carried out at both 3 and 14 days post-NPX to evaluate the renal responses to acute HS administration early in the adaptation to renal mass reduction,

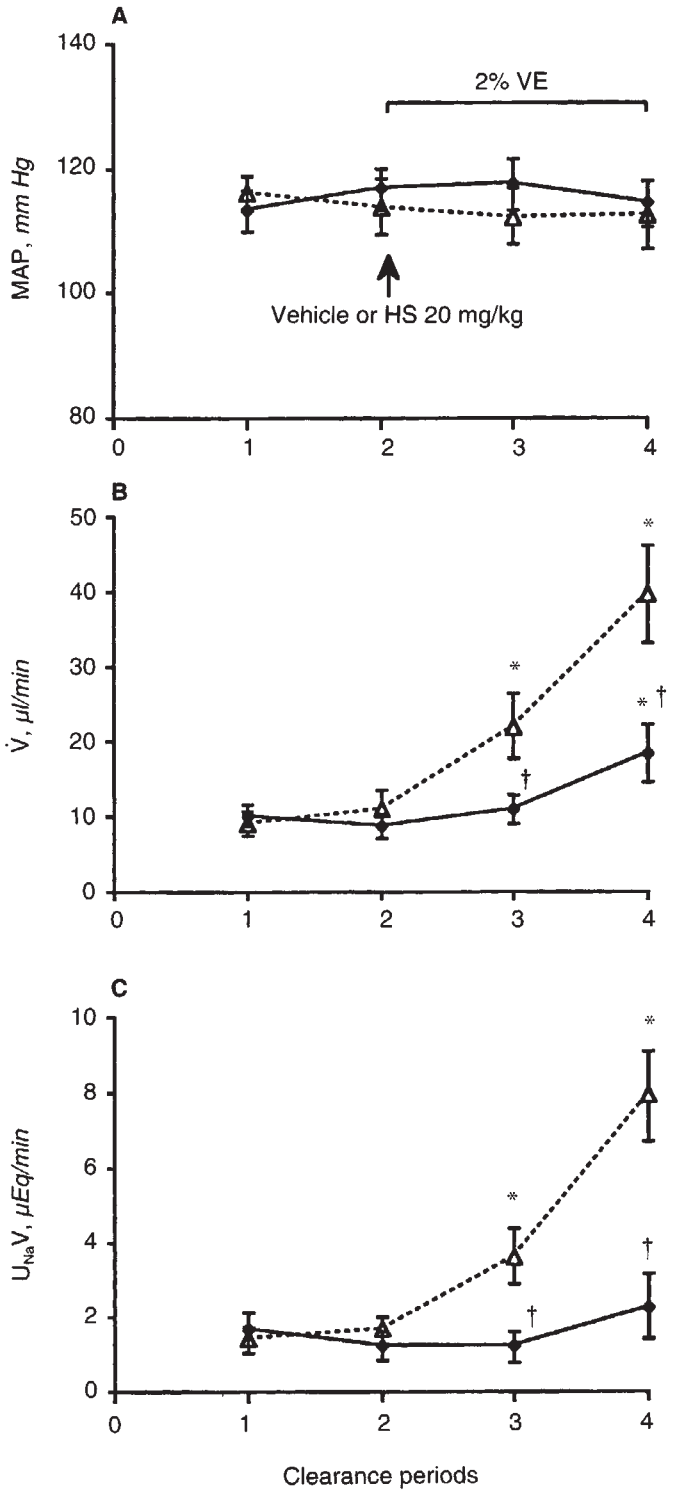


Fig. 2. A. *Mean blood pressure (MAP), (B) urine flow rate (V), and (C) urinary sodium excretion rate ($U_{Na}V$) before and during acute volume expansion in vehicle (···△···) or HS treated (—◆—) normal rats.* Values represent mean ± SE. *$P < 0.05$ vs. baseline and †$P < 0.05$ vs. vehicle treated normal rats in each group.

when functional responses might be expected to predominate, and later, when structural hypertrophy is also likely to contribute to the overall adaptive response.

Three days post-NPX

There were no significant differences in body weight among high (262 ± 8 g, $N = 15$), normal (262 ± 8 g, $N = 12$), and low (253 ± 8 g, $N = 13$) sodium rats. During both baseline and HS treatment periods, MAP was not significantly different between vehicle and HS-treated rats at each level of sodium intake (Table 2). Only high sodium rats showed a significant elevation in MAP after HS, whereas MAP in the other groups remained unchanged. Baseline values for GFR and ERPF were similar in all groups. HS administration was associated with sustained, significant reductions in GFR in high (36%) and normal (14%) sodium rats. In low sodium rats, on the other hand, GFR was not significantly changed following HS. ERPF was significantly reduced in high sodium rats following HS, but was not significantly changed in either normal or low sodium rats. Both V and $U_{Na}V$ were reduced by 70 to 80% in high sodium rats and by 50 to 65% in normal sodium rats. In marked contrast, however, neither V nor $U_{Na}V$ was altered significantly following HS in rats on low salt intake. Fractional excretion of sodium (FE_{Na}) observed during baseline periods in high sodium rats was twofold higher than in normal sodium rats and sevenfold higher than in low sodium rats. After HS treatment, however, FE_{Na} in high and normal sodium rats fell markedly, indicating that the observed decrease in $U_{Na}V$ was due to increased tubule reabsorption as well as decreased filtered sodium load. In contrast, in rats on the low sodium diet, FE_{Na} was less dramatically altered. Hematocrit (Hct) was decreased a small but significant amount after HS only in high sodium rats; in normal and low sodium rats Hct was unchanged. Under high, normal and low dietary sodium intake levels, the above indices of renal excretory and hemodynamic function were all unchanged following vehicle administration in time control groups (Table 2). Changes in GFR, $U_{Na}V$ and FE_{Na} after HS are demonstrated in Figure 3 at each sodium intake level.

Fourteen days post-NPX

There were no significant differences in body weight among high (252 ± 9 g, $N = 14$), normal (269 ± 5 g, $N = 14$), and low (259 ± 11 g, $N = 12$) sodium rats. During both baseline and HS treatment periods, MAP was not significantly different between vehicle and HS treated rats at the various level of sodium intake (Table 3). MAP was significantly elevated in high and normal sodium rats after HS whereas MAP was unaltered in low sodium rats after HS. Similar baseline levels of GFR and ERPF were observed among all groups. HS administration was associated with significant reductions in GFR of 56, 22 and 14% in high, normal and low sodium rats, respectively. After HS, ERPF was also significantly reduced by 51% in high sodium rats, but was unchanged in the low and normal sodium rats. Filtration fraction (FF) was significantly reduced after HS, in high sodium rats from 0.29 ± 0.01 to 0.26 ± 0.03 and in normal sodium rats, from 0.28 ± 0.01 to 0.21 ± 0.02, whereas FF was not significantly changed (0.23 ± 0.01 to 0.21 ± 0.02) in low sodium rats. As in the 24 hour studies, basal levels of $U_{Na}V$ under anesthesia also reflected levels of dietary sodium intake. After HS treatment, V was reduced by approximately 84% and 60% in high and normal sodium rats, respectively (Table 3). Similarly striking decreases in $U_{Na}V$ were observed in high (92%) and normal (81%) sodium rats. In low sodium rats, whereas V was not significantly changed following HS, a small but significant reduction in $U_{Na}V$ was observed. As

Table 1. Glomerular filtration rate (GFR), effective renal plasma flow rate (ERPF), filtration fraction, fractional excretion of sodium (FE_{Na}), and hematocrit (Hct) before and during volume expansion in vehicle and HS treated groups of protocol A

		GFR	ERPF	Filtration fraction	FE_{Na}	Hct
		ml/min			%	
Vehicle group	Baseline	1.10 ± 0.09	5.49 ± 0.58	0.21 ± 0.02	1.05 ± 0.26	45.3 ± 0.6
$N = 6$	VE	1.21 ± 0.09[a]	6.79 ± 0.59[a]	0.18 ± 0.02[a]	3.48 ± 0.59[a]	42.2 ± 0.7[a]
HS group	Baseline	1.12 ± 0.03	5.23 ± 0.42	0.21 ± 0.02	0.91 ± 0.26	46.5 ± 0.9
$N = 7$	VE	1.06 ± 0.08	6.19 ± 0.45[a]	0.18 ± 0.01[a]	1.18 ± 0.44[b]	42.6 ± 1.2[a]

Abbreviations are: Baseline, average of collection periods 1 and 2; VE, average of volume expansion periods (periods 3 and 4); HS, 20 mg/kg, i.v. bolus.

[a] $P < 0.05$ vs. baseline in each group

[b] $P < 0.05$ vs. vehicle group

Table 2. Mean arterial pressure (MAP), glomerular filtration rate (GFR), effective renal plasma flow rate (ERPF), urine flow rate (V), sodium excretion rate ($U_{Na}V$), fractional excretion of sodium (FE_{Na}) and hematocrit (Hct) in response to vehicle or HS in euvolemic rats 3 days after the 5/6 nephrectomy on high, normal (Nor) and low sodium intakes

		MAP *mm Hg*		GFR *ml/min*		ERPF *ml/min*		V *μl/min*		$U_{Na}V$ *μEq/min*		FE_{Na} %		Hct %	
	N	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
High-V[a]	7	125 ± 6	130 ± 6	0.57 ± 0.04	0.60 ± 0.06	2.07 ± 0.17	2.23 ± 0.15	28.3 ± 3.7	28.0 ± 2.6	4.0 ± 0.6	3.9 ± 0.4	5.7 ± 0.8	5.5 ± 0.6	47 ± 1	47 ± 1
High-HS[b]	8	136 ± 6	142[c] ± 6	0.55 ± 0.05	0.35[cd] ± 0.04	1.83 ± 0.19	1.53[c] ± 0.16	31.1 ± 4.8	9.4[cd] ± 2.1	5.5 ± 0.9	0.9[cd] ± 0.3	7.0 ± 1.1	1.5[cd] ± 0.4	46 ± 2	44[c] ± 2
Nor-V	6	121 ± 4	129 ± 3	0.49 ± 0.06	0.47 ± 0.07	2.05 ± 0.23	2.01 ± 0.31	16.4 ± 4.1	16.1 ± 3.6	2.1 ± 0.7	2.0 ± 0.6	2.6 ± 0.7	2.8 ± 0.7	46 ± 1	45 ± 1
Nor-HS	6	116 ± 3	131 ± 3	0.43 ± 0.05	0.37[c] ± 0.05	2.12 ± 0.33	2.07 ± 0.38	16.4 ± 4.1	8.2[cd] ± 1.7	2.0 ± 0.6	0.7[cd] ± 0.2	3.0 ± 0.7	1.3[cd] ± 0.4	47 ± 2	46 ± 2
Low-V	6	116 ± 7	122 ± 7	0.55 ± 0.07	0.53 ± 0.06	1.96 ± 0.24	1.86 ± 0.21	14.8 ± 3.0	13.9 ± 1.9	0.55 ± 0.26	0.51 ± 0.18	0.72 ± 0.32	0.69 ± 0.22	50 ± 1	50 ± 1
Low-HS	7	113 ± 6	121 ± 5	0.51 ± 0.11	0.56 ± 0.10	2.17 ± 0.56	2.32 ± 0.8	13.6 ± 2.7	10.4 ± 1.9	0.53 ± 0.19	0.27 ± 0.11	1.18 ± 0.58	0.63 ± 0.28	47 ± 3	46 ± 3

Values are means ± SE.

[a] V, vehicle

[b] HS, 20 mg/kg

[c] $P < 0.05$ vs. pre value within each group

[d] $P < 0.05$ vs. vehicle group

with $U_{Na}V$, baseline values for FE_{Na} reflected levels of dietary salt intake. After HS treatment, FE_{Na} was significantly decreased by approximately 83% and 78% in high and normal sodium rats, respectively. In low sodium rats, 70% decreases in FE_{Na} were also observed. HS administration was associated with significant reductions in hematocrit in high and normal sodium rats but not in low sodium rats. Following vehicle administration, FF was unchanged in high (0.25 ± 0.02 vs. 0.26 ± 0.02), normal (0.30 ± 0.02 vs. 0.31 ± 0.02) and low (0.24 ± 0.02 vs. 0.22 ± 0.03) sodium rats. None of the other indices of renal excretory and hemodynamic function was altered significantly in the time-control rats on high, normal or low sodium intakes (Table 3). Changes in GFR, $U_{Na}V$ and FE_{Na} after HS at various levels of sodium intake are shown in Figure 3.

Discussion

HS-142-1, a polysaccharide fermentation product of *Aureobasidium sp,* is a specific antagonist of natriuretic peptides, which competes for binding sites at guanylyl cyclase-linked natriuretic peptide receptors [11, 12]. HS has been shown to block the actions of the structurally related natriuretic peptides BNP, urodilatin and C-type natriuretic peptide [13, 14]. While earlier studies have shown that the diuretic and natriuretic responses to volume expansion may be blocked by specific anti-ANP anti-serum [15], a pharmacologic blocker of natriuretic peptide receptors provides a more precise tool for assessing the contribution of all NP to the functional adaptations to renal mass reduction. In protocol A we established that acute administration of HS markedly blunted the diuretic and natriuretic responses to acute saline loading in the euvolemic Münich-Wistar rat, confirming a previous report of similar findings in Sprague-Dawley rats, restricted to assessment of V and electrolyte excretion only [6]. Our data show that both renal components of the natriuretic response, that is, increases in filtered load and decreased tubule reabsorption of sodium, are blocked by HS. That the diuretic and natriuretic responses to acute saline loading are not completely abrogated in either study is not altogether surprising, since several other factors, such as reduced activity of renal sympathetic nerves, inhibition of vasopressin release, decreased renin-angiotensin system activity, and peritubular physical factors are also thought to contribute to the natriuretic response to acute saline loading [16, 17]. The degree to which FE_{Na} and natriuresis is blunted, however, is remarkable, suggesting that natriuretic peptides are factors of prime importance in mediating the acute renal responses to saline loading in anesthetized rats, at least over the time period observed in our study. In previous studies, we found that, using the same dose of HS (20 mg/kg) in normal euvolemic rats, GFR was reduced by

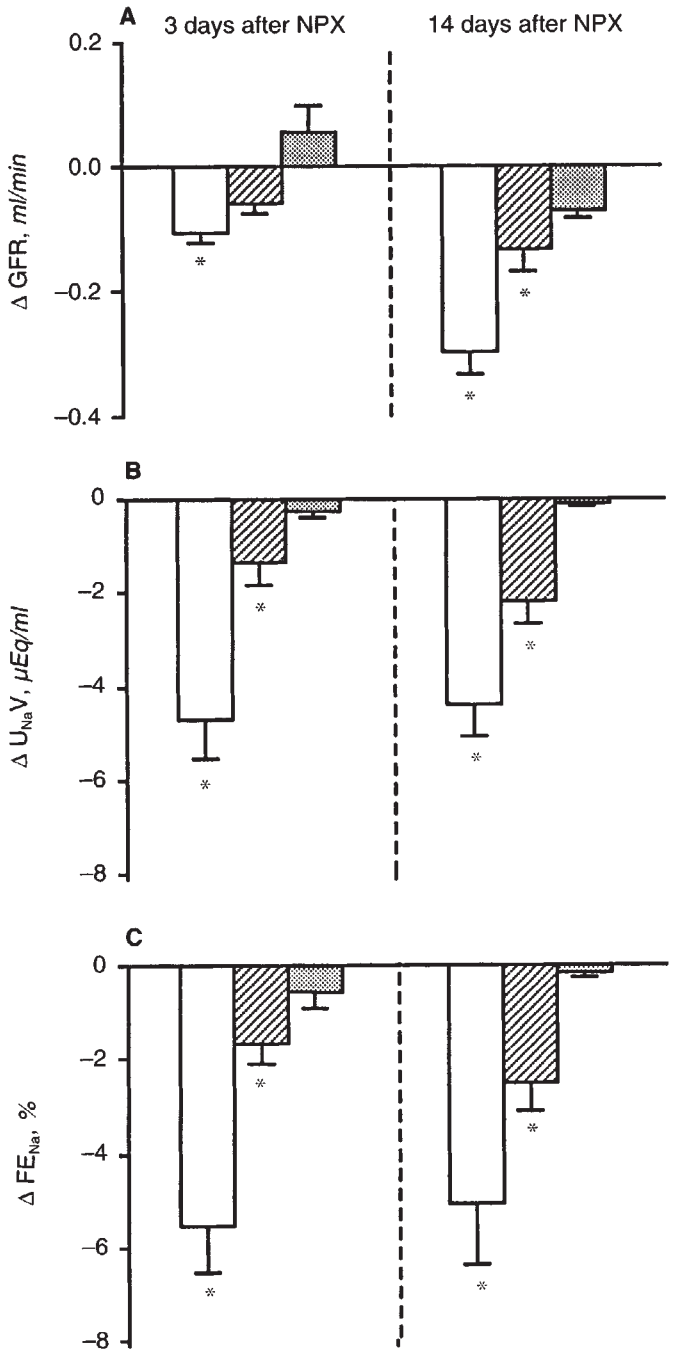


Fig. 3. *Changes (Δ) in: (A) glomerular filtration rate (GFR), (B) sodium excretion rate ($U_{Na}V$), and (C) fractional sodium excretion (FE_{Na}) following HS in rats 3 or 14 days after 5/6 nephrectomy (NPX), at each level of sodium intake.* Symbols are: (□) high sodium; (▨) normal sodium; (▩) low sodium. Values represent mean ± SE. *$P < 0.05$ vs. changes in GFR, $U_{Na}V$ and FE_{Na} after vehicle administration, respectively.

20% but was unchanged under hydropenic conditions [18], suggesting that the renal hemodynamic responses to HS are determined by changes in volume status and therefore natriuretic peptide activity. These studies also show that HS lacks nonspecific effects on GFR.

In experimental models of chronic renal failure, a reduction in functioning renal mass leads to retention of sodium and water which contributes to the development of systemic and glomerular capillary hypertension, adaptations which ultimately prove detrimental to the remnant kidney [1, 19]. One adaptive mechanism to counteract this tendency to excessive fluid retention is a decrease in sodium and water reabsorption by the renal tubules, as reflected in higher FE_{Na} in the remnant kidney [1]. In protocol B, the reductions in $U_{Na}V$ and FE_{Na} from elevated to near-normal levels following blockade of the natriuretic receptor, strongly suggest that natriuretic peptides contribute in large part to the elevated FE_{Na} in sodium replete NPX rats, at both 3 and 14 days after NPX. These observations are concordant with the data on plasma ANP levels and the responses to anti-ANP antiserum in NPX Münich-Wistar rats obtained under comparable experimental conditions in previously published studies from this laboratory [2, 3]. These data showed that plasma ANP levels were elevated in NPX rats on high sodium intake, but decreased in response to dietary sodium restriction. As in our present study, the reductions in $U_{Na}V$ and FE_{Na} observed in rats on low sodium diet following anti-ANP antibody were of markedly lesser magnitude than in those on high salt diet [2]. Taken together, these observations provide strong evidence that natriuretic peptides are of critical importance in mediating the adaptive decreases in tubule sodium reabsorption that occur in the remnant kidney in response to renal mass reduction under sodium-replete conditions. While the previously obtained radioimmunoassay data clearly implicate atrial natriuretic peptide in contributing to these adaptive functional responses, our current HS data leave open the possibility of a contributory role for other structurally related natriuretic peptides; identification of their relative contributions is the subject of work in progress in this laboratory.

In addition to alterations in tubule sodium and volume reabsorption, the other adaptive mechanism which promotes renal excretion of sodium and water is increased GFR per remnant nephron and hence increased filtered load of sodium. Glomerular hyperfiltration in NPX rats at 3 days is due largely to increased glomerular capillary pressure, which is in turn determined by preferential afferent arteriolar vasodilation and increased glomerular blood flow [1]. The mechanism whereby exogenous ANP, when administered in high doses, increases GFR is similar: preglomerular vasodilation, small or negligible increments in efferent arteriolar resistance, and elevation of the ultrafiltration coefficient (K_f) [20]. Our present data concerning whole kidney GFR and ERPF in NPX rats indicate that blockade of natriuretic peptide receptors with HS reduced GFR in rats on high or normal salt intake at 14 days, as well as at 3 days post-NPX. Interestingly, the level to which GFR was reduced in high salt rats at 3 days is close to the value that may be anticipated from 5/6 reduction in total filtration capacity, assuming no adaptive increase in remnant function takes place (approximately 0.35 ml/min). ERPF was also reduced significantly in rats on high salt intake, but not on normal salt intake. In contrast, both GFR and ERPF were unaffected by HS in rats on low salt intake. At 14 days, the data clearly demonstrate that natriuretic peptides contribute, to a large extent, to the maintenance of both GFR and ERPF under sodium-replete conditions. If anything, the renal hemodynamic responses to HS are greater at 14 days than at 3 days: a 56% reduction in GFR versus 36% at 3 days, and a 51% reduction in ERPF versus 17% at 3 days. This interesting observation suggests that, with time, the

Table 3. Mean arterial pressure (MAP), glomerular filtration rate (GFR), effective renal plasma flow rate (ERPF), urine flow rate (V), sodium excretion rate ($U_{Na}V$), fractional excretion of sodium (FE_{Na}) and hematocrit (Hct) in response to vehicle or HS in euvolemic rats 14 days after 5/6 nephrectomy on high, normal (Nor) and low sodium intakes

		MAP *mm Hg*		GFR *ml/min*		ERPF *ml/min*		V *μl/min*		$U_{Na}V$ *μEq/min*		FE_{Na} *%*		Hct *%*	
	N	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
High-V[a]	6	138 ± 15	149 ± 9	0.62 ± 0.04	0.61 ± 0.04	2.29 ± 0.17	2.21 ± 0.16	32.7 ± 7.2	35.6 ± 7.1	5.5 ± 1.4	6.1 ± 1.4	5.7 ± 1.1	6.6 ± 1.5	39 ± 2	38 2
High-HS[b]	8	135 ± 8	151[c] ± 8	0.52 ± 0.05	0.22[cd] ± 0.03	1.79 ± 0.23	0.83[cd] ± 0.22	29.0 ± 3.6	4.6[cd] ± 0.6	4.8 ± 0.7	0.36[cd] ± 0.1	6.2 ± 1.3	1.1[cd] ± 0.2	36 ± 2	33[cd] ± 2
Nor-V	6	146 ± 12	140[c] ± 9	0.56 ± 0.05	0.50 ± 0.04	1.87 ± 0.29	1.47 ± 0.10	26.3 ± 4.8	24.4 ± 4.6	2.9 ± 0.9	2.6 ± 0.8	3.8 ± 1.3	3.7 ± 1.3	41 ± 2	40 2
Nor-HS	8	149 ± 3	154 ± 5	0.66 ± 0.07	0.52[cd] ± 0.06	2.34 ± 0.23	2.51 ± 0.31	24.7 ± 3.8	9.6[cd] ± 0.8	2.7 ± 0.5	0.54[cd] ± 0.1	3.2 ± 0.6	0.7[cd] ± 0.1	39 ± 2	37[c] ± 2
Low-V	6	122 ± 8	125 ± 8	0.49 ± 0.05	0.41 ± 0.04	2.074 ± 0.30	1.96 ± 0.31	10.8 ± 2.1	10.2 ± 31.7	0.23 ± 0.16	0.15 ± 0.09	0.39 ± 0.31	0.25 ± 0.16	45 ± 1	45 ± 1
Low-HS	6	125 ± 9	130 ± 7	0.51 ± 0.05	0.44[c] ± 0.05	2.18 ± 0.21	2.16 ± 0.26	6.1 ± 1.0	4.5 ± 2.3	0.15 ± 0.08	0.05[c] ± 0.01	0.23 ± 0.13	0.07[c] ± 0.02	42 ± 1	41 1

Values are means ± SE.
[a] V, vehicle
[b] HS, 20 mg/kg
[c] $P < 0.05$ vs. pre value within each group
[d] $P < 0.05$ vs. vehicle group

maintenance of remnant kidney function becomes increasingly dependent upon the activity of natriuretic peptides.

These findings are at variance with our earlier report showing that while FE_{Na} was reduced by anti-ANP antiserum, GFR in high salt NPX rats was unaffected [3]. This may be due to the relatively low doses of ANP antiserum used, or alternatively, to differences in accessibility of these agents to their target sites. While immunoglobulin may be well suited to binding and thus inactivating circulating ANP, by virtue of its high molecular weight its access to natriuretic peptides generated within the kidney itself may be restricted. HS, on the other hand, a relatively low molecular weight polysaccharide, may be expected to have greater tissue penetrance, thus gaining access to natriuretic peptide receptors within the renal parenchyma.

Interestingly, as reported previously [3, 21], basal levels of GFR and ERPF between NPX rats on high or low salt diet are not significantly different. This observation suggests that factors other than natriuretic peptides serve to sustain glomerular hyperfiltration and hyperperfusion in this model during salt restricted conditions. Possible candidates include angiotensin, prostaglandins and nitric oxide. Tubule reabsorption of sodium, on the other hand, appears to be more sensitive to the effects of HS, even under the condition of salt restriction [18]. This is in keeping with the overall tendency for the renal tubules to be more responsive to small changes in natriuretic peptide activity than the determinants of renal hemodynamics. In our previous studies neither GFR nor FE_{Na} was reduced under hydropenic conditions. Thus, it is possible that the reductions in FE_{Na} observed in the low salt groups in this study reflect the effects of euvolemic preparation. Alternatively, activity of locally produced natriuretic peptides, such as urodilatin, may not be entirely suppressed under low sodium conditions.

In experimental animals, sustained glomerular hypertension has been shown to produce injury to the constituent cells of the glomerulus resulting in the histopathologic picture of focal and segmental glomerular sclerosis and hyalinosis [1, 19]. Ultimately, functioning nephrons are lost, thus reinforcing the stimulus to compensatory hypertrophy and hyperfunction acting on remnant nephrons. Since dietary sodium restriction in NPX rats has been demonstrated to reduce glomerular injury [21], suppression of natriuretic peptide activity in the kidney could also possibly limit the development of structural injury in NPX rats. Since our present data indicate an important role for natriuretic peptides in maintaining elevations in GFR and FE_{Na} per nephron under salt-replete conditions, chronic administration of HS to NPX rats may serve to suppress the development of maladaptive glomerular hemodynamic responses and thereby retard, or even abolish the ultimate development of glomerulosclerosis. These chronic studies await synthesis of the suitably large quantities of HS needed to perform them.

In summary, we confirm that HS inhibits the diuretic and natriuretic responses to acute volume expansion in normal rats. These effects of HS on renal excretory function may be largely explained by inhibition of the known pharmacologic effects of ANP or other natriuretic peptides on the renal glomerulus and tubules. In NPX rats we now show that under sodium replete conditions, GFR and FE_{Na} are markedly reduced following HS administration, but not in rats on low salt intake. These data suggest that natriuretic peptides mediate the compensatory increases in GFR, ERPF and tubule sodium excretion in the remnant kidney under sodium-replete conditions, and that dietary sodium restriction, which prevents volume expansion and therefore the increase in ANP secretion, prevents or ameliorates these effects.

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